IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of Aviv Shaish

Serial No.	10/668,601	Group Art Unit: 1654
Filed:	09/24/2003	Examiner: Michele C. Flood
For:	THERAPEUTIC USES OF *DUNALIELLA* POWDER	

DECLARATION
under Rule 132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

I, Ami Ben-Amotz, an Israeli citizen residing at 21 Hatomer St., Savyon, Israel, hereby declare:

1. I am currently Senior Investigator at The National Institute of Oceanography, Israel Oceanographic & Limnological Research, Haifa, Israel.

2. My list of publications was previously furnished in this matter as an Annex to my declaration of September 5, 2004 (hereinafter: "*my 2004 declaration*"). My fields of expertise include Algal biochemistry; Carotenoids biosynthesis; Separation and identification of cartenoids and retinoids.

3. I am familiar with the above captioned application (hereinafter: "*the application*"), and with the claims thereof.

4. I am also familiar with the Examiner's office communication of December 8, 2004, and in particular with the Examiner's comments regarding the articles described in my 2004 declaration.

5. The Office Action is based in part on a hypothesis from the prior art, i.e. that atherogenesis involves oxidative modification of LDL, which is associated with the depletion of the LDL endogenous anti-oxidants, and that enrichment of LDL with the anti-oxidant β-carotene has the potential of reducing the susceptibility of LDL to lipid peroxidation. However, as stated in my 2004 declaration, "*in recent years, and certainly by the filing date of the application (late 2003), this hypothesis had been proven to be in error* (para. 7)."

6. Early work in this field is exemplified by the following articles: Abbey M, Nestel PJ, Baghurst PA. *Antioxidant vitamins and low-density-lipoprotein*

oxidation. Am. J. Clin. Nutr. 1993;58:525-532 (Annex A); Reaven PD, Khouw A, Beltz WF, Parthasarathy S, Witztum JL: *Effect of dietary antioxidant combinations in humans. Protection of LDL by vitamin E but not by beta-carotene*. Arterioscler. Thromb. 1993; 13:590-600 (Annex B); and my own work, described in Levy, et al.: *Effect of Dietary Supplementation of Different Beta-carotene Isomers of Lipoprotein Oxidative Modification*. J. Nut. Env. Med. 1995; 5:13-22 (reference W in the Notice of References Cited) and in Levy, et al.: *Dietary Supplementation of a Natural Isomer Mixture of Beta-carotene Inhibits Oxidation of LDL Derived from Patients with Diabetes Mellitus*. Ann. Nut. Metab. 2000; 44(2):54-60 (reference U in the Notice of References Cited). A common characteristic of each of these four early articles is that the experiments performed relate to *in vitro* testing of LDL samples derived from patients; no *in vivo* data was collected or discussed. From this *in vitro* data, it was predicted that certain *in vivo* patient outcomes would result. It was this prediction, that the *in vitro* data would be predictive of *in vivo* clinical outcomes, that was shown in several later articles to be incorrect.

7. Later work in this field is exemplified by the following articles: Yusaf, S. et al, *Vitamin E supplementation and cardiovascular events in high-risk patients*, New England Journal of Medicine (2000) 342:154-60 [Annex C; treatment with an anti-oxidant such as vitamin E has no apparent effect on cardiovascular (CV) outcomes]; Kritharides, L. and Stocker, R., *The use of antioxidant supplements in coronary heart disease*, Atherosclerosis (2002) 164:211-21 [Annex D; supplements of vitamin E and β-carotene cannot be recommended for the treatment or prevention of Coronary Heart Disease]; Zureik, M. et al, *Effects of long-term daily low-dose supplementation with antioxidant vitamins and minerals on structure and function of large arteries*, Arterioscler. Thromb. Vasc. Biol. (2004) 24:1485-1491 [Annex E; observing no beneficial effects of supplementation with antioxidant vitamins and minerals on carotid atherosclerosis]; Jialal, I and Devaraj, S. Circulation (2003) 107:926-928 (Annex F; "*However, results of prospective antioxidant clinical trials have been disappointing*" (page 926, left-hand column, 2nd paragraph). "*It is clear that the antioxidant cocktails have no benefit in the prevention of CVD*" (page 928, left-hand column, third full paragraph)]; Clarke, R. and Armitage, J. (2002)

Antioxidant vitamins and risk of cardiovascular disease. Review of large-scale randomized trials. Cardiovascular Drugs and Therapy, 16:411-415 [Annex G; the findings from 3 large-scale trials of β-carotene supplementation involving 70,000 people and 5 large-scale trials of vitamin E supplementation involving 29,000 patients failed to confirm any protective effect for cardiovascular disease]; and Hegele, R.A., *ACE inhibition in the secondary prevention of vascular disease: the Heart Outcomes Prevention Evaluation (HOPE) trial and its substudies*, Current Atherosclerosis Reports (2000) 2:361-362 [Annex H; vitamin E supplementation had no apparent effect on cardiovascular outcomes].

8. It is apparent from an analysis of the results of large population *in vivo* testing described above, at the date of the filing of the application it had become clear that the hypothesis on which the Levy and other early articles were based was incorrect, and the Levy articles had been shown to be in error with respect to the effect of the use of β-carotene obtained from crude *Dunaliella* powder as an antioxidant in the treatment of diabetes mellitus and atherosclerosis. In view of the findings that antioxidants lack efficacy in the treatment of cardiovascular disease, scientists working in the field would have disregarded the conclusions of the Levy articles.

9. Thus, based on the large-scale clinical studies showing that vitamin E and β-carotene supplementation does not have a therapeutic effect on cardiovascular disease, in 2003 it would have been expected by one of ordinary skill in the art that crude *Dunaliella* powder would not have a therapeutic effect as a result of its anti-oxidant content (the active agent in the crude *Dunaliella* powder used in the invention is β-carotene; see the paragraph bridging pages 1 and 2 of the Specification). It was thus unpredictable and not obvious at the time this application was filed that crude *Dunaliella* powder would have an effect on atherosclerosis, and it was surprising and unexpected that crude *Dunaliella* powder has a therapeutic effect as taught by the present invention.

10. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 2, 2005 Ami Ben-Amotz

Prof. Ami Ben-Amotz